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BIOSYNTHESIS OF TYLOPHORINE AND TYLOPHORININE

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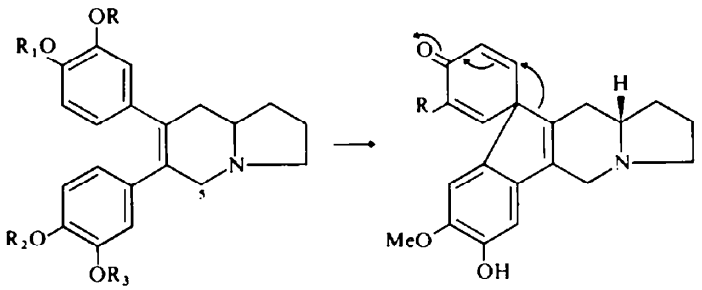
Abstract Administration of 3,4-dihydroxyphenyl[2-^{14}C] alanine to young *Tylophora asthmatica* plants revealed that ring B and carbon atoms C_9 and $C_{7'}$ of tylophorine and tylophorinine are derived from dopa. Tracer experiments with 6,7-diphenylhexahydroindolizines (**1**–**7**) and (**26**) demonstrated that compound **1** is efficiently and specifically incorporated into tylophorine (**13**) and tylophorinine (**16**). Compounds (**3**), (**4**) and (**26**) were not metabolized by the plants to form (**13**) and (**16**) whereas (**5**) and (**6**) were utilized to yield (**13**) and (**16**). Compound (**2**) was very poorly converted into (**13**) and (**16**) and thus is not on the major biosynthetic pathway of (**13**) and (**16**).

Phenanthroindolizidine alkaloids, tylophorine (**13**)[1,2] and tylophorinine (**16**)[1,3] are considered to be formed in nature by oxidative coupling[4] of suitably substituted 6,7-diphenylhexahydro-indolizine precursors. Herbert and Jackson[5] have recently reported intact incorporation of (**1**) and (**27**) into tylophorine (**13**), tylophorinine (**16**) and tylophorinidine in *Tylophora asthmatica* Further the keto acids (**17**), (**18**) and (**19**) were also found satisfactory precursors of tylophorinine (**16**).[6] However, the sites of labelling in the derived alkaloid were not established. Based on these results the major biosynthetic pathway to (**1**) was suggested as (**21**) → (**22**) → (**23**) → (**27**) → (**1**).

It has been postulated earlier[7–10] that condensation of 3,4-dihydroxyphenacylpyrrolidine (**24**) with 3,4-dihydroxyphenylpyruvic acid can give the intermediate of the type (**28**) which can undergo oxidative coupling to give tylophorine (**13**) skeleton. Since 3,4-dihydroxybenzoylacetic acid, 3,4-dihydroxyphenylpyruvic acid and Δ^1-pyrroline (**20**) can be derived in nature from phenylalanine, 4-hydroxy-phenylalanine and ornithine respectively. Mulchandani *et al.*[9,10] in accordance with the theory demonstrated that ring A and carbon atoms C_{10} and C_6, and ring B and carbon atoms C_9 and $C_{7'}$, of tylophorine (**13**) in *T. asthmatica* plants are derived from phenylalanine and tyrosine respectively. Ornithine was also incorporated, thus suggesting its participation in tylophorine biosynthesis via Δ^1-pyrroline (**20**). Since no degradation was carried out to determine the position of radio label the possible role of ornithine as a precursor of the pyrrolidine ring in tylophorine and tylophorinine at present is based on the analogy of results obtained in other plants.

3,4-Dihydroxyphenylpyruvic acid, the suggested precursor of ring B and carbon atoms C_9 and $C_{7'}$ of *Tylophora* alkaloids can also derive in nature by deamination of 3,4-dihydroxyphenylalanine. If this route is being followed in the plants, the trisubstituted 6,7-diphenylhexahydroindolizine of the type (**27**) cannot be an intermediate in the major biosynthetic pathway of these alkaloids. Instead, a tetrasubstituted 6,7-diphenylhexahydroindolizine of the type (**5**) should be a key intermediate. The phenolic hydroxyl groups of (**5**) can selectively methylate to direct the coupling. Tylophorine (**13**) and tylophorinine (**16**) can thus form in nature from suitably substituted 6,7-diphenylhexahydroindolizine precursors by alternate biosynthetic pathways as follows: *para-para*-oxidative coupling of (**1**) as suggested[5] can give the key dienone intermediate (**9**) which can undergo dienone-phenol rearrangement to form tylophorine skeleton (**12**) whereas dienone-benzene rearrangement can afford tylophorinine skeleton (**14**). Tylophorinine (**16**) can then form from (**14**) by hydroxylation at C_6 and methylation of the phenolic hydroxylic groups. In the second possibility *para-para* coupling of (**3**) can yield the dienone (**29**). Dienone-phenol rearrangement as shown in (**29**) followed by methylation of the phenolic hydroxyl groups can finally give tylophorine (**13**). In the third possibility direct *para-para* oxidative coupling of (**2**) can give tylophorine skeleton (**12**). Both tylophorine (**13**) and tylophorinine (**16**) can also form in nature from trisubstituted 6,7-diphenylhexahydroindolizine (**26**). *Para-Para*-oxidative coupling of (**26**) can form the dienone (**10**). Dienone-phenol rearrangement as shown in (**10**) can give tylophorinine skeleton (**15**). Tylophorine (**13**) can form from (**15**) by hydroxylation in the aromatic ring followed by methylation of the phenolic hydroxyl groups.

Tyrosine (Experiment 1) and phenylalanine (Experiment 2), the established precursors of tylophorine (**13**) and tylophorinine (**16**) were initially fed to young *T. asthmatica* plants. It was found that the alkaloids of interest were being biosynthesized by the plants. The experiment was repeated by feeding DL-[2-^{14}C] tyrosine (Experiment 3). The biosynthetic tylophorine (**13**) was degraded according to the procedure of Mulchandani *et al.*[9] It was found that essentially all the ^{14}C activity in the biosynthetic base was resided at position 7', thus confirming the earlier results.[9] 3,4-Dihydroxyphenyl[2-^{14}C] alanine (Experiment 4) was then fed to young *T. asthmatica* plants and after 8 days the biosynthetic tylophorine (**13**) and tylophorinine (**16**) were isolated by reverse dilution technique. The regiospecificity of the label in the biosynthetic tylophorine (**13**) was established as follows. Treatment of labelled tylophorine (**13**) with



(1) $R_1 = R_3 = H$; $R = R_2 = Me$
(2) $R = R_3 = H$; $R_1 = R_2 = Me$
(3) $R = R_2 = H$; $R_1 = R_3 = Me$
(4) $R = R_1 = R_2 = R_3 = Me$
(5) $R = R_1 = R_2 = R_3 = H$
(6) $R = Me$; $R_1 = R_2 = R_3 = H$
(7) $R_2 = Me$; $R = R_1 = R_3 = H$
(8) $R = R_2 = Me$; $R_1 = R_3 = Bz$

(9) R = OMe
(10) R = H

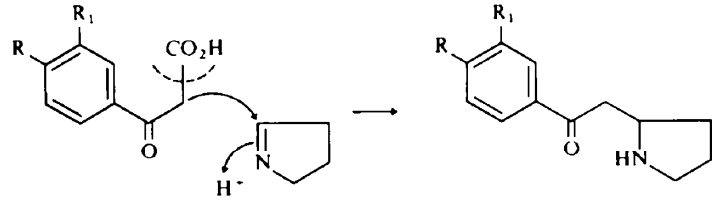
(11)

(12) R = H
(13) R = Me

(14) $R = R_2 = H$; $R_1 = Me$
(15) $R = H$; $R_1 = R_2 = Me$
(16) $R = OH$, $R_1 = R_2 = Me$

methyliodide afforded tylophorine methiodide (**36**) with essentially the same radioactivity as the parent base. Emde degradation of radioactive (**36**) gave isodihydrohomotylophorine (**39**) (Emde base) with practically no loss of radio activity. Kuhn–Roth oxidation of radioactive (**39**) gave radioactive acetic acid which was converted by Schmidt reaction into radioactive methylamine, isolated as N-methylbenzamide (92.5% original activity). The result thus established that ring B and carbon atoms C_9 and C_7 in tylophorine (**13**) are derived from 3,4-dihydroxyphenylalanine in young *T. asthmatica* plants.

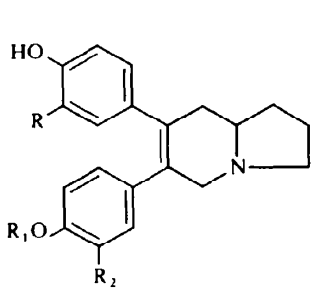
The position of label in the biosynthetic tylophorinine (**16**) derived from [2-^{14}C]dopa was established as follows: Treatment of biosynthetic tylophorinine (**16**) with methyliodide gave radioactive



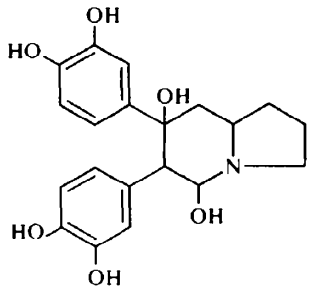
(17) $R = R_1 = H$
(18) $R = OH$; $R_1 = H$
(19) $R = OH$; $R_1 = OMe$

(20)

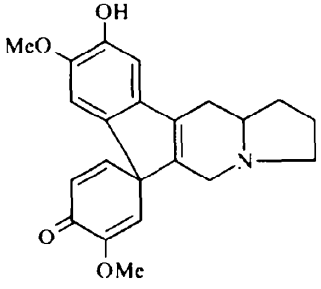
(21) $R = R_1 = H$
(22) $R = OH$; $R_1 = H$
(23) $R = OH$; $R_1 = H$
(23) $R = OH$, $R_1 = OMe$
(24) $R = R_1 = OH$
(25) $R = OBz$; $R_1 = OMe$



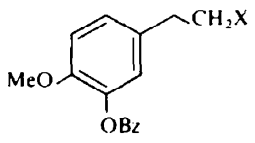
(26) R = H; R_1 = Me; R_2 = OH
(27) R = OMe; R_1 = R_2 = H



(28)

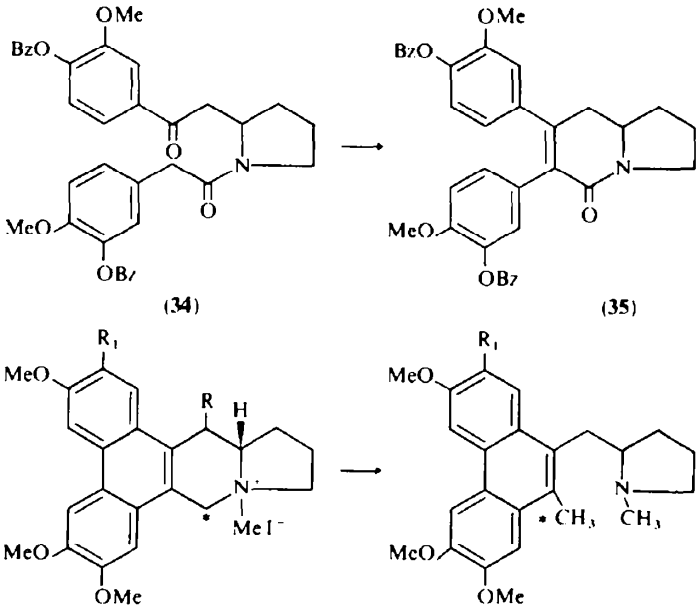


(29)



(30) X = Cl
(31) X = CN
(32) X = CO_2H
(33) X = COCl

tylophorinine methiodide (**38**) with essentially the same radioactivity as the parent base. Emde degradation of (**38**) gave isodihydrohomodeoxytylophorinine (**40**) with essentially no loss of radioactivity. Isotetrahydrohomodeoxytylophorinine was also obtained as reaction product, albeit in low yield. Isodihydrohomodeoxytylophorinine (**40**) was also prepared from (**16**) by another route. Hydrogenolysis of (**16**) in the presence of Pd/C afforded deoxytylophorinine (**15**). Treatment of (**15**) with methyliodide yielded the methiodide (**37**) and Emde degradation of (**37**) then afforded (**40**). Kuhn–Roth oxidation of radioactive (**40**) gave radioactive acetic acid which was converted by Schmidt reaction into radioactive



(34) (35)

(36) R = H; R_1 = OMe
(37) R = R_1 = H
(38) R = OH; R_1 = H

(39) R_1 = OMe
(40) R_1 = H

Kuhn-Roth

$C_6H_5CONH\overset{*}{C}H_3$ ← $\overset{*}{C}H_3NH_2$ ← $\overset{*}{C}H_3CO_2H$

methylamine, isolated as *N*-methylbenzamide (97.7% original activity). The result thus established that ring B and carbon atoms C_9 and $C_{7'}$ in tylophorinine (**16**) are derived from 3,4-dihydroxyphenylalanine.

The late stages of biosynthesis of tylophorine (**13**) and tylophorinine (**16**) were studied by feeding tritium and ^{14}C labelled 6,7-diphenylhexahydroindolizines to young *T. asthmatica* plants. Hypothetical 6,7-diphenylhexahydroindolizine precursors were synthesized by the method of Herbert *et al.*[11] 2-Phenacylpyrrolidines were conveniently prepared by condensation of Δ^1-pyrroline with appropriate benzoylacetic acids. Treatment of 2-phenacylpyrrolidines with appropriate phenylacetaldehydes afforded the corresponding enamines which were reduced *in situ* with sodium borohydride to give the protected 6,7-diphenylhexahydroindolizines. Acid catalysed hydrogenolysis of the benzyl ethers finally afforded the corresponding hydroxy compounds. Following this procedure 6,7-diphenylhexahydroindolizines (**1**), (**2**), (**3**), (**4**) and (**26**) were prepared. Tritium specifically *ortho*- and *para*- to hydroxyl groups were introduced by base catalysed exchange reaction.[12] 6,7-Diphenylhexahydroindolizines (**1**), (**5**), (**6**) and (**7**) labelled with ^{14}C at position 5 were prepared as follows: Treatment of appropriate benzyl chloride with $K^{14}CN$ afforded labelled benzyl nitriles. Alkaline hydrolysis of the nitriles yielded 3,4-disubstituted phenyl [2-^{14}C] acetic acids which were converted into the corresponding acid chlorides. Condensation of the acid chlorides with appropriate 2-phenacylpyrrolidines yielded the keto-amides of the type (**34**). Treatment of (34) with ethanolic KOH gave the lactum (**35**). Lithium aluminium hydride reduction of (**35**) in the presence of $AlCl_3$ gave the protected 6,7-diphenylhexahydroindolizine (**8**). Acid catalysed debenzylation of (**8**) finally yielded the corresponding hydroxy compound (**1**) labelled with ^{14}C at position 5.

Labelled hypothetical 6,7-diphenylhexahydroindolizine precursors were fed to young *T. asthmatica* plants. The results of several feedings are recorded in Table 1. Feedings of (±)-(**3**) (Experiment 9) and (±)-(**26**) (Experiment 8) showed that (**3**) and (**26**) were not metabolized by the plants to form (**13**) and (**16**). As expected, the completely methylated 6,7-diphenylhexahydroindolizine (**4**) (Experiment 10) was not utilized by the plants to form (**13**) and (**16**). Feedings of (±)-6-(3-hydroxy-4-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,2,3,5,8,8a-hexahydroindolizine (**1**) (Experiment 6) and (±)- (**2**) (Experiment 11) demonstrated that (**1**) was very efficiently incorporated into tylophorine (**13**) and tylophorinine (**16**) in *T. asthmatica*. (2) was metabolized by the plants very poorly.

The regiospecificity of the label in biosynthetic tylophorine (**3**) derived from the feeding of (**1**) was established by the degradation procedure of Mulchandani *et al.*[9] *N*-Methylbenzamide, so obtained, had 95.4% original activity. The result thus established that essentially all the ^{14}C activity at position 5 in (**1**) was resided at position 7' in biosynthetic tylophorine (**13**).

The position of label in biosynthetic tylophorinine (**16**) derived from (**1**) was located by the above procedure. The *N*-methylbenzamide, obtained by the procedure had 96.5% original activity. The result thus established that essentially all the ^{14}C activity of (**1**) at position 5 was present at position 7' in biosynthetic tylophorinine (**16**). The foregoing experiments confirmed the results of Herbert *et al.*[5] and established that both tylophorine (**13**) and tylophorinine (**16**) in *T. asthmatica* are specifically biosynthesized from (**1**).

Efficient and specific incorporation of 3,4-dihydroxyphenylalanine (dopa) in ring B of tylophorine (**13**) and tylophorinine (**16**) in *T. asthmatica* plants and that of (**5**) into both the alkaloids supported the biogenetic theory that 3,4-dihydroxylphenacylpyrrolidine (**24**) and 3,4-dihydroxyphenylpyruvic acid are condensed to form the tetrahydroxy intermediate (**5**). The key intermediate (**1**) can form from (**5**) *via* (**6**) or (**7**). Parallel feedings of (±)-(**6**) (Experiment 12) and (±)-

Table 1. Tracer experiments on *T. asthmatica*

Expt.	Precursor fed	% Incorporation × 10^2	
		Tylophorine/Tylophorinine	
		(13)	(16)
1	(DL)-[U-^{14}C] Tyrosine	0.33	
2	(±)-3-Phenyl[2-^{14}C] alanine	2.6	2.9
3	(±)-[2-^{14}C] Tyrosine	3.4	2.1
4	(±)-[2-^{14}C] Dopa	4.2	2.7
5	[3',5'-$^3H_2$] 4-Hydroxyphenyl [3-$^3H_2$] pyruvic acid	0.6	0.65
6	(±)-6-(3-Hydroxy-4-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-hexahydro (5-^{14}C] indolizine (1)	13.1	4.2
7	(±)-[5-^{14}C] Indolizine (5)	2.60	1.40
8	(±)-[3',5',2'',6''-$^3H_4$] Indolizine (26)	0.012	Inactive
9	(±)-[2',6',3''-$^3H_3$] Indolizine (3)	0.06	Inactive
10	(±)-[2',6',2'',6''-$^3H_4$] Indolizine (4)	0.05	0.02
11	(±)-[2',6',2'',6''-$^3H_4$] Indolizine (2)	2.00	0.52
12	(±)-[5-^{14}C] Indolizine (6)	1.22	0.30
13	(±)-[5-^{14}C] Indolizine (7)	0.05	0.01

(7) (Experiment 13) revealed that selective *O*-methylation of (5) gives (6) which is then converted into (1).

The foregoing experiments strongly suggest the biosynthetic pathways of tylophorine (13) and tylophorinine (16) in *T. asthmatica* as follows: Phenylalanine + Dopa → (5) → (6) → (1) → tylophorine (13) and tylophorinine (16).

EXPERIMENTAL

For general directions (spectroscopy details and counting method) see Reference 12.

Synthesis of precursors. 6,7-Diphenylhexahydroindolizines (1), (2), (3), (4) and (26) were prepared according to the method of Herbert *et al.*[11] (1), (5), (6) and (7) labelled with ^{14}C at position 5 were synthesized by the route as exemplified by the synthesis of (1).

Synthesis of (±)-6-(3-hydroxy-4-methoxyphenyl-7-(4-hydroxy-3-methoxyphenyl)-1,2,3,5,8,8a-hexahydro-[5-^{14}C]-indolizine (1)

3-Benzyloxy-4-methoxybenzyl nitrile (31). (30) (300 mg) in dry DMSO (3 ml) was added to a suspension of $K^{14}CN$ (activity = 0.05 mCi) in dry DMSO (2 ml) and stirred (24 h). Inactive KCN (200 mg) was added to complete the reaction and worked up in the usual manner to afford radioactive *nitrile* (31), m.p. 78–80° (lit.[13] 79.5–80.5°).

Specific activity = 0.072×10^{-2} mCi/mg

3-Benzyloxy-4-methoxyphenylacetic acid (32). The radioactive (31) (245 mg) in glycol (10 ml) was refluxed with KOH (210 mg) in H_2O (2 ml) for 20 h to give radioactive acid (32) (200 mg), m.p. 123° (lit.[13] 125–26°), crystallized from C_6H_6–petroleum ether to constant activity.

Specific activity = 7.0×10^{-4} mCi/mg.

3-Benzyloxy-4-methoxyphenylacetyl chloride (33). The radioactive (32) (200 mg) in dry C_6H_6 (5 ml) was refluxed with $SOCl_2$ (0.15 ml) for 2 h to give the radioactive acid chloride (33).

2-(4-Benzyloxy-3-methoxyphenacyl)pyrrolidine (25). An aqueous solution (pH ~ 7.0) of Δ^1-pyrroline[14] (20) (0.28 g) was added dropwise to an ice cooled solution (pH ~ 7.0) of 4-benzyloxy-3-methoxybenzoylacetic acid (1.2 g) [prepared from corresponding ethyl ester[15] by alkaline hydrolysis] in MeOH (25 ml) and phosphate buffer (pH ~ 7.0, 50 ml) (N_2 atmosphere). The resulting mixture was left for 48 h at ambient temperature, acidified with 10% HCl and extracted with ether. The aqueous acidic solution was basified with 28% NH_4OH and the liberated base was extracted with ether, washed with H_2O, dried (Na_2SO_4) and the solvent removed. The residue, so obtained, was chromatographed over Al_2O_3. Elution with C_6H_6–$CHCl_3$ (1:1) afforded (25) (0.65 g, 52%), m.p. 195–96° (Me_2CO), IR (neat), 3200 (–NH) and 1660 cm^{-1}, NMR ($CDCl_3$): 7.51–7.1 (7 H, m, Ar–H), 6.74 (1 H, d, J = 9 Hz, Ar–H), 5.05 (2 H, s, $-OCH_2$–Ar), 3.83 (3 H, s, OCH_3), 2.98 (4 H, m, N–CH_2– and CO–CH_2–Ar). (Found: C, 73.60; H, 7.75; N, 4.15. $C_{20}H_{23}NO_3$ requires: C, 73.84; H, 7.77; N, 4.37%.)

2-(4-Benzyloxy-3-methoxyphenacyl)-N-(3-benzyloxy-4-methoxyphenylacetyl)pyrrolidine (34). (33) (200 mg) in dry C_6H_6 (5 ml) was added to a solution of (25) (270 mg) in dry C_6H_6 (5 ml) and pyridine (0.05 ml) and left at room temperature for 20 h. The product, so formed, was filtered, washed with 1% HCl, H_2O, dried (Na_2SO_4), IR (neat) 2950, 1670, 1635, 1595 and 1510 cm^{-1}. The product was used as such for the next step.

6-(3-Benzyloxy-4-methoxyphenyl)-7-(4-benzyloxy-3-methoxyphenyl)-5-oxo-1,2,3,5,8,8a-hexahydroindolizine (35). (34) was refluxed with 5% ethanolic KOH (30 ml) for 3 h. The resulting mixture was worked up in the usual manner. The crude product, thus obtained, was chromatographed over SiO_2. Elution with $CHCl_3$–MeOH (99.5:0.5) gave the lactum (35) (230 mg), m.p. 124–25° ($CHCl_3$–petroleum ether); IR (KBr) 2930, 1635, 1600, 1505 and 1435 cm^{-1}; NMR ($CDCl_3$) 3.92 (3 H, s, $-OCH_3$), 3.83 (3 H, s, OCH_3), 5.1 (2 H, s, OCH_2–Ar), 5.16 (2 H, s, OCH_2–Ar), 7.5–7.1 (11 H, m, Ar–H). (Found: C, 77.00; H, 6.20; N, 2.41. $C_{36}H_{35}NO_5$ requires: C, 77.05; H, 6.23; N, 2.49%.)

6-(3-Benzyloxy-4-methoxy)-7-(4-benzyloxy-3-methoxyphenyl)-1,2,3,5,8,8a-hexahydroindolizine (8). (35) (220 mg) in tetrahydrofuran–ether (1:1, 10 ml) was added to an ice cooled suspension of LAH (200 mg) and $AlCl_3$ (200 mg) in dry ether (50 ml) and stirred at room temp. for 14 h. The resulting mixture was worked up in the usual manner to give the crude product which was purified by t.l.c. on silica gel plates (solvent: $CHCl_3$–MeOH, 97:3) to give the *amine* (8) (162 mg), m.p. 131–32° ($CHCl_3$–petroleum ether); IR (KBr) 2880, 1590, 1510 and 1250 cm^{-1}; NMR (CCl_4): 7.1 (10 H, m, Ar–H), 4.8 (2 H, s, OCH_2–Ar), 4.6 (2 H, s, OCH_2–Ar), 3.65 (3 H, s, OCH_3) and 3.42 (3 H, s, OCH_3). (Found: C, 78.70; H, 6.62; N, 2.41. $C_{36}H_{37}NO_4$ requires: C, 78.97; H, 6.76; N, 2.55%.)

6-(3-Hydroxy-4-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,2,3,5,7,8a-hexahydroindolizine (1). (8) (160 mg) in MeOH (5 ml) was heated on a water bath with 1 N HCl (2.5 ml) for 2 h. The resulting mixture was worked up in the usual way to give base (1) hydrochloride (70 mg). Free base, m.p. 259–60° ($CHCl_3$–MeOH); λ_{max} (MeOH) 242, 258, 288 nm; λ_{max} (MeOH–NaOH) 237, 259, 291 nm; ν_{max} (KBr) 3300, 2900, 1600, 1590 and 1500 cm^{-1}; *m/e* 367 (M^+). (Found: C, 72.04; H, 6.72; N, 3.69. $C_{22}H_{25}NO_4$ requires: C, 71.93; H, 6.81; N, 3.81%.)

Labelling of Precursors

Tritiation. (±)-6,7-*Di*(3-hydroxy-4-methoxyphenyl)-1,2,3,5,8,8a-hexahydroindolizine (2) (75 mg) in tritiated water (0.3 ml; activity 70 mCi) containing potassium t-butoxide (150 mg) was heated under nitrogen (sealed tube) for 110 h at 100°. The mixture was diluted with H_2O, NH_4Cl was added (pH 7) and the liberated base was extracted with $CHCl_3$ (4 × 10 ml). The extract was washed with H_2O, dried (Na_2SO_4) and evaporated. The crude product was chromatographed on a column of SiO_2. Elution with $CHCl_3$–MeOH (92:8) gave (±)-2′,6′,2″,6″-$^3H_4$-6,7-*di*(3-*hydroxy*-4-*methoxyphenyl*)-1,2,3,5,8,8a-*hexahydroindolizine* (2) (52 mg), m.p. 227–28° (MeOH). The radiochemical purity of the sample was checked by dilution method. (±)-[2′,6′,3″-$^3H_3$]-6-(4-Hydroxy-3-methoxyphenyl)-7-(3-hydroxy-4-methoxyphenyl)-1,2,3,5,8,8a-hexahydroindolizine-(3) and (±)-3′,5′,2″,6″-$^3H_4$]-6-(3-Hydroxy-4-methoxyphenyl)-7-(4-hydroxyphenyl)-1,2,3,5,8,8a-hexahydroindolizine-(26) were prepared similarly (±)-[2′,6′,2″6″-$^3H_4$]-6,7-Di(3,4-dimethoxyphenyl)-1,2,3,5,8,8a-hexahydro-indolizine (4) was prepared from (2) by treatment with CH_2N_2.

(±)-6,7-Di(3,4-dihydroxyphenyl)-1,2,3,5,8,8a-hexahydro-[5-^{14}C]-indolizine (5), (±)-6-(3-hydroxy-4-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-1,2,3,5,8,8a-hexahydro-[5-^{14}C]-indolizine-(7) and (±)-6-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,2,3,5,8,8a-hexahydro-[5-^{14}C]-indolizine (6) were prepared by complete synthesis.

Feeding experiments. For feeding purposes tyrosine, dopa, phenylalanine, indolizine (1), (5), (6) and (7) hydrochlorides were dissolved in H_2O (2 ml). Indolizine precursors (2), (3), (4) and (26) were dissolved in H_2O (2 ml) containing DMSO (0.1 ml). The solutions of the precursors were fed to young *T. asthamatica* plants (1–2 yr old) by wick feeding method. The plants were kept alive for 8 to 10 days for metabolism, and then worked up for tylophorine and tylophorinine.

Isolation of tylophorine (13) *and tylophorinine* (16). Young *T. asthmatica* plants (120–160 g wet wt) were macerated in ethanol (300 ml) with radioinactive tylophorine (100 mg) and tylophorinine (100 mg) and left for 24 h. The ethanol was decanted and the plant material was percolated with fresh ethanol (containing 2% AcOH) (10 × 100 ml). The solvent from the combined percolate was removed under reduced

pressure to give the greenish viscous mass which was extracted with 5% HCl (6 × 25 ml). The aqueous acidic solution was defatted with ether (5 × 25 ml) and basified with aqueous $NaHCO_3$. The liberated bases were extracted with $CHCl_3$ (5 × 25 ml), washed with water, dried (Na_2SO_4) and solvent removed to give a crude mixture of tylophorine and tylophorinine (265 mg). The crude mixture was subjected to t.l.c. over SiO_2 plates (solvent: $CHCl_3$–MeOH, 96:4). The regions containing tylophorine and tylophorinine were cut and eluted with $CHCl_3$–MeOH (4:1). Removal of the solvent from the eluates gave *tylophorine* (**13**) (54 mg), m.p. 285–86° ($CHCl_3$–MeOH) (lit.[1b] 286–87°) and *tylophorinine* (**16**) (45 mg), m.p. 246–47° ($CHCl_3$–MeOH) (lit.[16] 248–49°). The isolated bases in feeding experiments were crystallized from $CHCl_3$–MeOH to constant activity. The radiochemical purity of the samples were checked by dilution technique.

Degradation of biosynthetic tylophorine derived from 3,4-dihydroxyphenyl [2-^{14}C] alanine. The biosynthetic tylophorine (**13**) (200 mg) in $CHCl_3$ (20 ml) was refluxed with MeI (2 ml) for 2 h and worked up in the usual manner to give radioactive *tylophorine methiodide* (**36**) (210 mg), m.p. 278° (H_2O) (lit.[1b] 280°). To a refluxing solution of radioactive (**36**) (200 mg) in MeOH–H_2O (1:9, 10 ml) was added Na–Hg (6 g) and then left overnight at ambient temperature. To the resulting mixture water (10 ml) was added, the product was extracted with C_6H_6, washed with H_2O, dried (Na_2SO_4) and evaporated to give radioactive isodihydrohomotylophorine (Emde base) (**39**) (80 mg), m.p. 202° (C_6H_6–petroleum ether) (lit.[17] 200–202°).

CrO_3 (5 g) in 2N H_2SO_4 (10 ml) was added dropwise to a solution of radioactive (**39**) (80 mg) in 2N H_2SO_4 (2 ml). The radioactive acetic acid thus formed was distilled, neutralized with 0.01 N NaOH and water removed to give radioactive sodium acetate (10 mg). It was diluted with radioinactive sodium acetate (35 mg). NaN_3 (100 mg) was added to a solution of radioactive sodium acetate in conc. H_2SO_4 (0.7 ml) at 0°. The hydrazoic acid and CO_2 evolved from the reaction mixture were removed by a current of N_2. The resulting mixture was heated at 70–80° for 1 h, cooled and basified with 10% NaOH. The liberated radioactive methylamine was distilled and received in 2N HCl (10 ml). The aqueous solution of radioactive methylamine hydrochloride was concentrated and treated with benzoyl chloride (0.2 ml) and 1N NaOH (8 ml). The product was extracted with ether, washed with H_2O, dried (Na_2SO_4) and the solvent removed to give radioactive *N-methylbenzamide* (8 mg), m.p. 78–79° (lit.[9] 78–79°). The radioactivities of the degradation products are given in Table 2.

Degradation of biosynthetic tylophorine derived from 6-(3-hydroxy-4-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,2,3,5,8,8a-hexahydro [5-^{14}C] indolizine. The biosynthetic tylophorine (**13**) (300 mg) in $CHCl_3$ (20 ml) was treated with CH_3I to give the radioactive *methiodide* (**36**) (312 mg). The radioactive (**36**) was degraded as above to give radioactive *N*-methylbenzamide. The radioactivities of the degradation products are given in Table 3.

Degradation of biosynthetic tylophorinine derived from 3,4-dihydroxyphenyl[2-^{14}C] alanine. The biosynthetic tylophorinine (**16**) (250 mg) in $CHCl_3$ (10 ml) was heated with CH_3I (2 ml) to give radioactive *tylophorinine methiodide* (**38**) (255 mg), m.p. 244–45° (decomp.) (MeOH) (lit.[16] 243–45°(d)). Radioactive (**38**) (250 mg) in EtOH (5 ml) and H_2O (5 ml) was refluxed with AgCl [prepared from $AgNO_3$ (0.5 g)] for 5 h and then left at room temp for 18 h. Ethanol from the resulting mixture was removed, the residue was taken in H_2O and heated. To the hot aqueous solution Na–Hg (12 g) was added. The reaction mixture was kept at room temp. for 20 h and worked up as earlier to give a crude product which was chromatographed on a column of basic alumina. Elution with C_6H_6 gave *isodihydrohomodeoxytylophorinine* (**40**) (36 mg), m.p. 180–81° (C_6H_6–hexane); *m/e* 379 (M^+); λ_{max} 257, 287 and 339 nm and 9-(*N-methylpyrrolidinyl-methyl*-10-*methyl*-9,10-*dihydro*-2,3,6-*trimethoxyphenanthrene* (3 mg), m.p. 160° (C_6H_6); *m/e* 381 (M^+); λ_{max} 256, 283 and 307 nm. (**40**) was also obtained by Emde degradation of deoxytylophorinine methiodide (**37**) which in turn was prepared from deoxytylophorinine (**15**)[16] by treatment with CH_3I.

Radioactive (**40**) (36 mg) was oxidised with CrO_3–2N H_2SO_4 as earlier to give radioactive *acetic acid* which was converted to *N*-methylbenzamide. The radioactivities of the degradation products are given in Table 4.

Table 4. Activities of degradation products of tylophorinine

Compound	Molar activity (disint. min^{-1} $mmol^{-1}$)
Tylophorinine (**16**)	2.59×10^4
Tylophorinine methiodide (**38**)	2.52×10^4
Emde base (**40**)	2.52×10^4
N-Methylbenzamide	2.53×10^4

Degradation of biosynthetic tylophorinine derived from 6-(3-hydroxy-4-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,2,3,5,8,8a-hexahydro 5-^{14}C indolizine. The biosynthetic tylophorinine (**16**) (290 mg) was converted into radioactive tylophorinine methiodide (**38**) and was degraded as above. The radioactivities of the degradation products are given in Table 5.

Table 5. Activities of degradation products of tylophorinine

Compound	Molar activity (disint. min^{-1} $mmol^{-1}$)
Tylophorinine (**16**)	4.80×10^4
Tylophorinine methiodide (**38**)	4.74×10^4
Emde base (**40**)	4.55×10^4
N-Methylbenzamide	4.63×10^4

Table 2. Activities of degradation products of tylophorine

Compound	Molar activity (disint. min^{-1} $mmol^{-1}$)
Tylophorine (**13**)	4.43×10^4
Tylophorine methiodide (**36**)	3.97×10^4
Emde base (**39**)	3.90×10^4
N-Methylbenzamide	4.1×10^4

Table 3. Activities of degradation products of tylophorine

Compound	Molar activity (disint. min^{-1} $mmol^{-1}$)
Tylophorine (**13**)	1.41×10^5
Tylophorine methiodide (**36**)	1.41×10^5
Emde base (**39**)	1.34×10^5
N-Methylbenzamide	1.35×10^5

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